Pilot TES

Status Report on Pilot Study
September 28, 2001

Clinical part is accomplished.

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100 %

Total number of subjects screened was 189. Total number of subjects who met inclusion/exclusion criteria and enrolled was 140. All informed consents have been obtained from enrolled subjects appropriately. Five subjects prematurely withdrew. Total number of subjects completing study was 135, which is exactly the number targeted in the Research Protocol. Case Report Forms (CRFs) audit has been completed by the monitor. All biological specimens, i.e., 24-hour urine, blood, and exhalate samples, have been shipped to the analytical laboratories.

Report and Analysis Plan (RAP) is accomplished.

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100 %

The plan provides for several strategies to examine the collected data. Univariate measures such as the frequency, mean (arithmetic and geometric), median, standard deviation, quantiles (5^{th} and 95^{th} percentiles), and range will be used to describe the study results. Separate analyses will be performed for each biomarker by smoking status (smoker and non-smoker). Comparisons between smokers and non-smokers will be made according to demographic variables using the t-test, U-test, or the chi-square test as indicated by the distribution of the data.

The data will be modeled using non-linear regression (Gompertz, asymmetric sigmoid shape) to characterize the relationship between the biomarkers and the estimated daily exposure response using machine-derived data and the number of cigarettes smoked daily. The FTC tar or the FTC CO values will be used with the assumption that all other smoke constituents are either proportional to tar (particle phase constituents) or to CO (gas phase constituents). Variables will be selected by using a regression tree approach.

SAS will be the primary statistical software used to analyze the data.

The evaluation of questionnaire, weekly survey, and diaries is in progress.

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30 %

The data from the pre-tested questionnaire, weekly survey, and diaries will enable us to verify subject inclusion, analyze and interpret "outliers" or unusual values in the data, evaluate aspects of smoking behavior, build dose-response model, and more accurately characterize exposure levels. Data verification and storage in the data bank is accomplished. Currently the CRO is constructing the "listing" program that will compile the data into the statistical tables as described in the RAP.

Jan Oey, September 28, 2001

Method development is accomplished.

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100 %

A total of 10 analytical methods were developed at Covance Laboratories in Madison and in Harrogate. These are: (1) LC-MS-MS to determine nicotine and metabolites in urine, (2) GC-TEA to determine NNAL and NNAL-glucuronide in urine, (3) GC-MS to determine 3- and 4-aminobiphenyl hemoglobin adduct in blood, (4) headspace GC-NPD to determine acetonitrile in blood, (5) thermal desorption GC-NPD to determine acetonitrile in exhalate, (6) HPLC with flourescence detection to determine malondialdehyde in blood, (7) HPLC with fluorescence detection to determine malondialdehyde in urine, (8) EIA immunoassay to determine 11-dehydro-thromboxane B2 in urine, (9) EIA immunoassay to determine 8-epi-prostaglandin-F2alpha in urine, and (10) HPLC to determine caffeine and metabolites in urine.
Two assays were established at the clinic and Covance Laboratory Services in Indianapolis: (1) electrochemical gas sensor to determine CO in exhalate, and (2) spectrophotometry (CO-Oximeter) to determine carboxyhemoglobin in blood.
In addition, there are 4 clinical analytical methods already established and validated at Covance Indianapolis: (1) enzymatic colorimetric assay to determine HDL- and LDL-cholesterol in blood, (2) photometric clot detection method to determine fibrinogen in blood, (3) a high-sensitive immunonephelometry assay to determine C-reactive protein in blood, and (4) alkaline picrate method to determine creatinine in urine.

Method validation is nearing completion

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80 %

The validation of the following 8 analytical methods is accomplished: (1) NNAL and NNAL-glucuronide in urine, (2) acetonitrile in blood, (3) 3- and 4-aminobiphenyl hemoglobin adducts in blood, (4) 11-dehydro-thromboxane B2 in urine, (5) 8-epi-prostaglandin-F2alpha in urine, (6) malondialdehyde in urine, (7) acetonitrile in exhalate, and (8) caffeine metabolites in urine . It is estimated that validation of the remaining 2 analytical methods will be completed in 2-3 weeks: (1) nicotine and metabolites in urine and (2) malondialdehyde in blood.

Sample analysis: 8 accomplished, 5 still in progress, and 3 not yet started.

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50 %

Accomplished: (1) CO in exhalate, (2) carboxyhemoglobin in blood, (3) acetonitrile in blood, (4) malondialdehyde in urine, (5) C-reactive protein in blood, (6) creatinine in urine, (7) HDL- and LDL-cholesterol in blood, and (8) fibrinogen in blood.
In progress: (1) NNAL and NNAL-glucuronide in urine, (2) 3- and 4-aminobiphenyl hemoglobin adduct in blood, (3) 11-dehydro-thromboxane B2 in urine, (4) 8-epi prostaglandin-F2alpha in urine, and (5) caffeine and caffeine metabolites in urine.
Not yet started: (1) nicotine and nicotine metabolites in urine, (2) acetonitrile in exhalate, and (3) malondialdehyde in blood.
The time line indicates that sample analysis will be completed on 11/09/01, when the analysis of NNAL and NNAL-glucuronide is accomplished.

Jan Oey, September 28, 2001

Publication of study design and biomarker selection is accomplished.

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100 %

Two posters on study design and on biomarker selection were presented at the International Congress of Toxicology in Brisbane, July 9, 2001. Abstracts were published.
In addition, these 2 posters plus 3 other posters on method validation, ETS assessment, and biomarkers of effects have been accepted to be presented at the symposium "Biomarker for Tobacco Exposure" in Minneapolis, October 24-26, 2001. However, the symposium will not publish the abstracts.

Open items:

- Evaluation of questionnaire, weekly survey, and diaries
- Method validation and validation report
- Sample analysis
- QA check of sample analysis data (biomarker data)
- Transfer of biomarker data to data base
- Evaluation of data base according to RAP
- Final report

Milestones:

• Method validation completed:	10/09/01
• Sample analysis completed:	11/09/01
• SAS programs finalized:	to be determined
• Database locked:	02/22/02
• Bio-analytical data available:	12/03/01
• Final report:	04/25/02

End of Status Report

Jan Oey, September 28, 2001